THE INFLUENCE OF EARLY LIFE ADVERSITY AND SOCIAL CUE ON COCAINE-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS

by

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The Influence of Early Life Adversity and Social Cue on Cocaine-Induced Dopamine Release in the Nucleus Accumbens

Thesis directed by Assistant Professor Sondra Bland.

ABSTRACT

The susceptibility to cocaine addiction varies as a function of idiosyncratic and environmental factors, differentially predisposing individuals to this disorder as a product of the combination of these variables. In particular, early life adversity has been identified as playing a key role in the development of substance abuse, and exposure to chronic stress during early critical periods may sensitise individuals to the reinforcing properties of psychostimulants. Furthermore, environmental factors greatly modulate the subjective experience of drug consumption, and the presence of a social cue may enhance the neurobiological response to drugs of reward. Specifically, the presence of a novel conspecific may elevate extracellular levels of dopamine in the nucleus accumbens, both as an intrinsically rewarding experience and as compensatory mechanism to the potential stress of the novel social exposure, thereby potentiating the action of the drug. Utilising the adolescent social deprivation (ASD) model, this study investigated the interactive effects of early life adversity and exposure to a novel social cue on the rewarding properties of cocaine, as measured by dopamine in the nucleus accumbens shell.

The form and content of this abstract are approved. I recommend its publication.

Approved: Sondra Bland

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CHAPTER I

INTRODUCTION

Cocaine is a potent psychostimulant which induces subjective feelings of euphoria, increased energy and increased confidence. However, due to its rewarding properties, it carries a high risk for addiction. According to figures provided on the National Institute of Drug Abuse (NIDA) website, 4.8 million Americans aged 12 and above had abused some form of cocaine in 2009.[1] As well as the medical risks posed by chronic abuse and acute overdose, there are socio-occupational issues which arise as a product of cocaine addiction. Individuals who abuse this drug may compromise their physiological and psychological health, create distress in their interpersonal relationships, and allow their cocaine habit to interfere with their professional careers. Crucially, however, cocaine affects individuals differentially, and a large proportion of people who experiment with this recreational drug do not develop maladaptive or destructive patterns of usage (Mugford, 1994; van der Poel, Rodenburg, Dijkstra, Stoele & van de Mheen, 2009). It is thus of great importance to identify and investigate those factors which predispose individuals to drug abuse and to apply them to our current understanding of the neurobiological basis of addiction.

Stress is acknowledged to play a key role in the reinforcing qualities of psychostimulants, and exposure to chronic stress may cause changes at the molecular level in the brain's reward pathways (Piazza & Le Moal, 1997; Andersen & Teicher, 2009). Adolescent social deprivation (ASD) provides a model of long-term stress which induces a range of aberrant neurological

[1] http://www.nida.nih.gov/drugpages/cocaine.html

outcomes (Fone & Porkess, 2008), and which can be utilised for the study of the development of psychostimulant sensitivity.

Environmental factors also strongly influence the valence of a given drug high, and inform the likelihood of developing future drug-seeking behaviour through their moderation of the subjective experience. In particular, exposure to a social cue through the presence of peers may increase extracellular dopamine levels, both as a coping mechanism for any social stress engendered by the novel conspecific, and as an intrinsic and adaptive reward derived from the play interaction. When paired with cocaine, this endogenous dopamine activity may create a potentiating effect that is highly reinforcing and predictive of future drug use. This study examined the interaction between the effects of adolescent social deprivation and exposure to a novel social cue on the reinforcing properties of cocaine, as measured by extracellular levels of dopamine in the nucleus accumbens, obtained in vivo by microdialysis.

Action of Cocaine

Cocaine exerts its action on the transmitters dopamine, serotonin and norepinephrine; however, this paper will focus exclusively on dopamine since it is this neuromodulator which has received the greatest attention in terms of cocaine's rewarding, addictive qualities.

Dopamine is one of the catecholamines found in the CNS and is involved in a wide range of behaviours, including motor function and reward. It is synthesised from tyrosine, an amino acid derived from food sources, which is converted to the precursor L-DOPA by the enzyme TH. L-DOPA, in turn, undergoes a conversion with L-aromatic amino acid decarboxylase, producing finally the monoamine dopamine (DA). Extracellular levels of dopamine are regulated by presynaptic autoreceptors in the cell membrane dispersed over many areas of the neuron,

receiving information in a negative feedback loop and adjusting the rate of tyrosine hydroxylation accordingly. Synaptic DA is also monitored by transporters located on the terminal boutons which perform reuptake, and are often found outside the synaptic cleft, regulating DA which may diffuse away. In the nucleus accumbens (NAcc), dopamine is metabolised by monoamine oxidase (MAO-A), an enzyme present in the membranes of mitochondria in terminal boutons and glia cells which breaks DA down into its organic compounds (Iversen, Iversen, Bloom & Roth, p. 186-191). DA follows two main pathways in the brain, the nigrostriatal pathway which originates in the substantia nigra of the midbrain and innervates the caudate nucleus and putamen (striatum), and the mesocorticolimbic pathway in which dopaminergic neurons in the ventral tegmental area project to the NAcc and cortical areas.

The nucleus accumbens is widely accepted as one of the brain's main centres for processing hedonic state and motivation (for a review see Berridge & Kringelbach, 2008), and increases of dopamine in this region are associated with reward. It is thought that this "pleasure" system evolved to reinforce evolutionarily advantageous behaviours, such as eating tasty food, performing sexual activity, and even engaging in social play (Berridge & Kringelbach; Trezza, Baarendse & Vanderschuren, 2010; Piazza et al., 1996). The aforementioned behaviours facilitate survival of the individual by supporting and encouraging the supply of nutrition to the body, the passing on of genes to future generations, and the adoption of appropriate social comportment through playful developmental interactions. It is here in the nucleus accumbens that cocaine exerts its influence (Roberts, Corcoran & Fibiger, 1977), essentially usurping the dopamine reward system with an artificial high, the mechanics of which are potentially problematic for the integrity of the NAcc and its dopaminergic innervations. The highly rewarding nature of the drug

encourages repeated consumption, however chronic cocaine usage results in the down-regulation of DA receptors, thereby cyclically promoting further use. In particular, while cocaine activates DA pathways in both the core and the shell of the NAcc, its rewarding properties are preferentially observed in the shell (Pontieri, Tanda & Di Chiara, 1995). The drug binds to and competitively inhibits the transporters which normally regulate the extracellular neurotransmitter through inhibiting synthesis and release, thereby increasing the amount of dopamine available in the synapse (Nestler, Hyman & Malenka, p.359).

Cocaine appears to facilitate DA binding at both D1 and D2 receptors, with some differentiation between the two in terms of the function of the dopaminergic response. In one human study (Haney, Ward, Foltin & Fischman, 2001), D1 receptors were implicated in both the subjective sensation of being 'high' and in the psychomotor, motivational response of self-administration. Support for the latter contention is found in a study conducted by Xu et al. (1994), in which mutant D1 receptor knock-out mice failed to show locomotor reactivity to a range of doses of cocaine whereas controls were unaffected, implicating the D1 subtype in psychomotor activity. Contrasting somewhat with findings of Haney et al. (2001), Chang, Sawyer, Lee and Woodward (1994) observed some subdivision of D1 and D2 receptors such that D1 types are implicated more in anticipatory behavioural responses, while D2 are implicated more in reinforcement (see also Maldonado et al., 1997). This is supported by findings that D1 neurons in the NAcc core are activated by exposure to contextual cues, thereby instigating self-administration (Fricks-Gleason & Marshall, 2011). Furthermore, Cain et al. (1997) suggest that the D2 receptor subtype D3 may be specifically involved in reinforcement and is therefore a likely contributor to the resulting formation of addiction. Thus while the findings on this subject

are not definitive or completely unambiguous, it does appear that there is a preferential delineation of dopamine receptor subtypes into an anticipatory/psychomotor response involving the D1 type and a rewarding, reinforcement response involving the D2 type.

Interestingly, there appears to be some dispute regarding the role of dopamine in our understanding of the processing of reward in the NAcc. In their review, Berridge and Kringelbach (2008) suggest that dopamine may be less involved in the sensation of pleasure itself, i.e. the hedonic experience, and that it instead acts as the motivating force which guides the individual or animal to engage in the rewarding behaviour. On the other hand, a study by Chang et al., (1994) found that while there is an anticipatory response in the NAcc of neuronal firing in rats approaching or engaging with a cocaine self-administration lever, these neurons were unaffected by either SCH 23390 or pimozide, a D1 and D2 antagonist respectively, raising the possibility that innervations other than the dopaminergic VTA to NAcc pathway may be involved in motivational instigation. Neurons which became activated post-cocaine administration were in fact affected by the antagonists, however, suggesting that dopamine is in fact integral to the drug-dependent subjective sensation of reward.

In sum, the release of dopamine and its prolongation in the synapse in the nucleus accumbens appears to be the locus of influence for cocaine and its habit-forming properties. Factors which facilitate or interact with this *modus operandi* may differentially predispose one individual over another to develop drug-seeking behaviour or addiction. The effects of environmental variables on the transmission of dopamine in the nucleus accumbens are therefore a potential source of vulnerability to psychostimulants and were thus examined as contributing risk factors.

Risk Factors

Environmental Influence

Contextual cues (such as the environment in which persons consume drugs) play an important role in producing sensitisation to and determining the valence of a given psychostimulant experience (Robinson, Browman, Crombag & Badiani, 1998), and can even induce craving by exposure to cues alone (Childress et al., 1999). In particular, the presence of a social cue has begun to receive attention as a possible moderating factor in the production of reward by drugs (Trezza, et al., 2010; Thiel, Okun & Neisewander, 2008). In their experiment, Thiel et al. administered low doses of cocaine (2mg/kg) to male rats and then paired them with a conspecific for a play session. Rats who received both cocaine and social cue showed conditioned place preference (CPP) for the interaction of these two variables, as measured by time preferentially spent in an environment contextually associated with the drug/social cue pairing. Control subjects, and subjects receiving either cocaine only or social cue only did not acquire CPP. This indicates that there may be a crucial potentiating effect which occurs as a product of the interaction between cocaine and social cue, creating a sensation of reward that goes over and above what either variable can produce in isolation. Thus for individuals who ingest psychostimulants in the company of friends, the risk of developing drug-seeking behaviour appears to be increased by the enhancement of the cocaine reward by the social reward. This is a most pertinent issue, as many young persons engage in cocaine consumption at social occasions (Mugford, 1994; van der Poel et al., 2009). It should be noted, however, that all subjects utilised by Thiel et al. were raised in the ASD model, and as such it is not possible to

determine whether the findings of a cocaine/social cue interaction are related to early social deprivation specifically, or whether such results are applicable to the general population.

Interestingly, the data collected by Thiel et al. run in some contrast to preliminary findings from our laboratory in which CPP was established for juvenile rats in the ASD condition exposed to social cue only, using the same experimental parameters outlined in the aforementioned study. In other words, in this pilot study we found that rats raised in social isolation are particularly susceptible to the reward engendered by exposure to a conspecific, and taken together with the (somewhat less robust) findings of Thiel et al., there is good reason to suppose that subjects in this condition may be at greater risk of developing a reward association that predicts future drug-seeking behaviour when cocaine and social cue are paired together. Thus environmental or social cues were investigated as a key factor in susceptibility to drug addiction.

Stress

Stress is widely accepted to play an important role in the development and maintenance of a variety of medical and psychological disorders, and is gaining increasing currency as a factor in the development of substance disorders. Indeed, stress has been implicated in the initiation, maintenance and relapse of drug addiction (Bland et al., 2004a; Bland, Schmid, Watkins & Maier, 2004b; Andersen & Teicher, 2009; Sinha, Catapano & O'Malley, 1999; Self & Choi, 2004). The mechanism responsible for this hyper-susceptibility to substance abuse is the activation of the stress-responsive hypothalamic-pituitary-adrenal (HPA) axis and its subsequent influence on DA neurons. During times of stress, the HPA axis is activated and glucocorticoids are released from the adrenal cortex; in humans this stress hormone is called cortisol, in rats it is corticosterone (CORT). Glucocorticoids can permeate the blood-brain barrier and are secreted

into the VTA which, as mentioned previously, richly innervates the mesocorticolimbic pathway. In this way glucocorticoids can exert influence on dopaminergic transmission in the NAcc. Although the findings regarding the precise mechanisms are not absolutely conclusive, it is thought that glucocorticoids act on three distinct stages of DA synthesis and regulation: they may enhance the synthesis of DA from tyrosine hydroxylase; they may decrease the enzymatic breakdown of dopamine by MAO; and they may inhibit reuptake of the neurotransmitter by the presynaptic transporters (Piazza & Le Moal, 1997; Andersen & Teicher, 2009). Conversely, administration of a CORT antagonist suppresses the stress-potentiated DA increase in response to morphine, further suggesting glucocorticoids' role in heightening the experience of reward (Der-Avakian et al., 2006). Thus the secretion of glucocorticoids greatly facilitates the level of extracellular dopamine in the NAcc, and therefore suggests that these stress hormones play an important modulatory role in the subjective experience of reward.

This stress-induced facilitation of DA release in the NAcc is most pertinent when the glucocorticoid secretion is coupled with a rewarding activity, such as consuming food or ingesting drugs like psychostimulants. Some reports suggest that DA levels in the NAcc increase to 80% above baseline when corticosterone administration is coupled with a pleasurable experience (Piazza & Le Moal, 1997), though not all findings support this effect in the absence of a true stressor (Der-Avakian et al., 2006). The positively reinforcing outcome of glucocorticoid secretion may be a compensatory device on the part of the organism to cope with environmental stressors, and coupled with the reward derived from a dose of cocaine there appears to be an interaction between the respective neurobiological responses, producing a greatly enhanced subjective sensation of pleasure. For individuals exposed to early chronic

stress, this interaction is amplified with more far-reaching effects. Long-term experience of stress increases the reactivity of the HPA axis, making the individual more susceptible to acute environmental stressors (Andersen & Teicher, 2009); by extension, sensitivity to drugs of addiction is increased through a heightened stress response and release of glucocorticoids, and the latters' subsequent facilitation of DA in the NAcc.

Thus the ingestion of cocaine under duress, or consumed by an individual exposed to chronic stress may be intrinsically more rewarding than ingestion in the absence of stress. Indeed, rats treated with corticosterone display greater cocaine-seeking behaviour than controls (Mantsch, Saphier & Goeders, 1998), and, interestingly, rats classified as highly responsive to stress are in fact more sensitive to corticosterone itself (Piazza et al., 1996). Taken together, these findings suggest that individuals with a pre-existing sensitivity to stress, or long-term exposure to negative life events, are more likely to develop addictive relationships with drugs as a product of their reactivity to stress-induced hormones, the latter figuring prominently in the acquisition of drug-seeking behaviour. Perhaps also the presence of stress provides greater motivation to self-medicate with rewarding substances as a means of ameliorating unpleasant feelings. Thus the role of stress in the potentiation of the reinforcing properties of cocaine was examined.

Early Life Adversity

Early life adversity is a key factor in the production of psychopathology, including substance disorders, as it induces chronic, long-term stress, the effects of which produce a range of changes in the developing individual. As mentioned above, exposure to stress may increase an individual's susceptibility to the rewarding effects of drugs of abuse by means of its modulation of the dopaminergic mesocorticolimbic pathway. Adolescent social deprivation (ASD) is a

widely recognised model of early life adversity, whereby the developing animal is denied social contact for a certain critical period of development. There is a wide consensus as to the importance of implementing this model during early, sensitive periods of development, and much work in this area brackets early weaning stages or the adolescent phase (Douglas, Varlinskaya & Spear, 2004; Ferdman, Murmu, Bock, Braun & Leshem, 2007; Hermes, Li, Duman & Duman, 2011; Weintraub, Singaravelu & Bhatnagar, 2010; Andersen, Lyss, Dumont & Teicher, 1999; Howes, Dalley, Morrison, Robbins & Everitt, 2000; Trezza, et al., 2010; Weiss, Domeney, Heidbreder, Moreau & Feldon, 2001; Thiel et al., 2008).

Exposure to ASD induces a range of abnormal behaviours, although the manner in which these anomalies manifest is a source of some contradiction. Some studies indicate that ASD reduces social interaction among rats (Hol, Van den Berg, Van Ree & Sprujit, 1999; Hermes et al., 2010), whereas others suggest that social interaction is significantly more rewarding for isolated subjects (Douglas et al., 2004; Ferdman et al., 2007). The increase in social interaction observed in some studies may be related to heightened aggression as a product of early life adversity (Fone & Porkess, 2008), though as the conclusions on this issue are not uniform more research must be done.

Preliminary results from our laboratory indicate CPP for access to a novel conspecific is increased in subjects raised in the ASD model compared to those raised in the group-housed condition. As discussed above, the postulated compensatory mechanism of the glucocorticoid-dopamine interaction suggests that the latter stance is more likely, with social cue a more intrinsically rewarding experience for subjects in the chronic isolation stress condition. Indeed, this is supported by the observation that rats raised in social isolation display greater motivation

toward obtaining food than normally raised controls (Fone & Porkess, 2008; Lomanowska et al., 2011), food reward being mediated by the same dopaminergic pathway that processes both social play and cocaine reward.

According to a review by Andersen and Teicher (2009), the early life stress of maternal separation causes significant and long-lasting changes to the regulation of monoamines in the NAcc, such that there is an increased basal level of dopamine and a decreased level of serotonin in the adult ASD rat compared with controls (see also Andersen et al., 1999; Higley & Linnoila, 1997). They hypothesise that this elevated basal level of DA may be responsible for a heightened predisposition to seek out drugs of reward like cocaine, as it sensitises the NAcc to the effects of psychostimulants by the reduction of DA transporters; some research suggests by as much as even 250% (Meaney, Brake & Gratton, 2002). There may also be a concurrent up-regulation of DA D3 receptors in the shell, further sensitising individuals to the reinforcing action of the drug (Brake, Zhang, Diorio, Meaney & Gratton, 2004). This is further reinforced by deficits in the prefrontal cortex (PFC), resulting in impaired ability to self-regulate behaviour.[2] Early life adversity may decrease DA and 5-HT activity in the PFC as it increases it in the NAcc (Fone & Porkess, 2008; Dalley, Theobald, Pereira, Li & Robbins, 2002), resulting in an individual with increased vulnerability to the reinforcing sensations of drugs like cocaine, and with decreased capability to monitor inappropriate usage. Interestingly, Howes et al. (2000) did not find a

[2] Adolescence, generally speaking, is a time of immaturity in terms of decision-making. A study by Ernst et al. (2005) demonstrates that teenagers, compared to adults, have greater activation of the NAcc and less activation of the amygdala when participating in the Iowa Gambling Task. This suggests that young persons focus more on potential benefits and less on potential risks, whereas adults take the latter into greater consideration. This reaction puts youth at large in a disadvantageous position when it comes to making decisions regarding drug use; this is likely potentiated by the acquired deficits of early life adversity discussed above.

significant increase in basal levels of dopamine, serotonin and glutamate in their ASD subjects, offering an inconsistency to the observations of Andersen and Teicher (see also Dalley et al., 2002). Importantly, however, the authors did find two noteworthy differences between groups; isolated subjects displayed greater cocaine-seeking than controls, as measured by lever presses in a self-administration paradigm (see also Meaney et al., 2002; Piazza & Le Moal, 1997); secondly, extracellular dopamine levels in the NAcc after administration of cocaine at 1.5mg/kg (with a trend also at 0.25mg/kg) was significantly higher for the ASD group. Thus while no significant variation was observed in basal neurotransmitter levels in this study, the behavioural outcome of interest and the pattern of dopamine response in the nucleus accumbens between groups do in fact support the argument of long-term neurobiological changes as a product of ASD, as suggested by Andersen and Teicher.

In terms of responsiveness to stress, early life adversity has a significant effect on the individual's reaction to both acute and chronic stressors, differentially stimulating the HPA axis between ASD and control groups (Weintraub et al., 2010). Although one study found no difference between ASD rats and controls in the behavioural response to the stress of a novel environment (Weiss et al., 2001), it is important to note that this test did not examine the impact of social stress on the animal. Whereas in the former study ASD rats were rehoused in groups for 20 days prior to experimentation, subjects in the latter experiment were not, thus is it conceivable that the housing conditions in the Weintraub et al. study provided a chronic stressor to ASD subjects and may have played an important role in the production of their differential stress responses where such an effect was absent in the non-social stress test of Weiss et al. Thus as a

mechanism of neurobiological change and mediator of stress response, the role of early life adversity in the modulation of cocaine reward was studied.

CHAPTER II

METHOD

Subjects

Sprague-Dawley rats (n = 76) were obtained from the Harlan breeding facility at post-natal day 21. The rats were contained in standard polycarbonate tub cages lined with aspen bedding, and had *ad libitum* access to food and water. Light in the animal colony room was programmed on a 12 hour dark/12 hour light cycle, and the temperature was maintained at a constant 27ºC.

Housing conditions

Immediately upon arrival in the animal facility, subjects were assigned to either isolated housing condition or group housing condition; the former were placed individually in their cages, while the latter were housed in a same-sex fashion 3 per cage. The rats remained in their respective housing conditions for 4 weeks prior to experimentation in order to bracket the developmental adolescent period of interest.

Procedures

All procedures detailed below were in accordance with guidelines set out by the Institutional Animal Care and Use Committee (IACUC) of the University of Colorado.

Surgical Procedures

After the subjects spent 4 weeks in their respective housing conditions they underwent surgery, during which a guide cannula was inserted into the NAcc shell (see Figure 1) using aseptic procedures. The guide cannula is a small sheath encasing a stylet which is lowered into the brain region of interest, and which provides a structured channel to guide the insertion of the microdialysis probe (discussed in more detail below) during experimentation. The animals were

anaesthetised with isofluorane, a gas-form barbiturate, and secured in the stereotaxic apparatus, a device which both restrains the subject's head and allows for the accurate location of the nucleus accumbens by means of coordinates (Paxinos & Watson, 1998). The correct entry point was located using coordinates in 2 planes, anterior-posterior (AP) and lateral-medial (LM). Bregma provided the point of reference on the skull, the point at which the AP and LM sutures intersect. The following calculations were then performed to obtain the location of the nucleus accumbens: Males, AP = bregma + 1.7mm, LM = bregma ± 0.8mm. Females, AP = bregma + 1.6mm, LM = ± 0.7mm. Addition/subtraction in the LM plane was alternated from subject to subject to obviate any potential hemispherical bias. Once these calculations were made and the coordinates for the NAcc obtained, an access hole for the cannula was drilled on the skull, exposing but not disturbing the surface of the brain. The depth of entry of the cannula was obtained from a third set of coordinates in the dorsal-ventral plane (DV). As with the AP and LM plotting, measurements were first obtained from a reference point, this time from the tip of the cannula resting lightly on the dura mater: Males, DV = -4.7; Females, DV = -4.6. The cannula was lowered and secured in place by fusing it to the skull with screws placed in the three quadrants not yet accessed, overlaid with dental acrylic cement. Animals were then administered both penicillin and carprofen through subcutaneous injection, an antibiotic and an analgesic, respectively. The dose of penicillin was uniform for each subject at 42,000 units in .14ml; the dose of carprofen was adjusted for each animal and was administered at 5mg/kg at an injection volume of 1ml/kg.

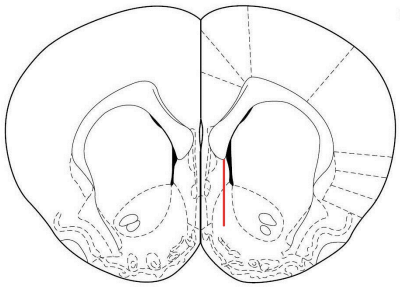

Figure I. Representative section of NAcc with probe placement.

Experimental Procedures

Subjects were allowed to recuperate for one week, with after-care shots of penicillin and carprofen administered once each day for two days post-surgery at the same respective doses outlined above. Then, five days after the guide cannula was implanted, the animals were handled for five minutes each in advance of the microdialysis procedure in order to habituate them to the necessary experimental manipulations. The following day the rats were moved to the experimental room and placed individually in clear Plexiglas boxes supplied with food, water and bedding, and fitted with a tether system which protects the microdialysis tubing. The subjects were then secured to the tether, at which point the stylet was removed and the microdialysis probe inserted. The 2mm probe tip is a semi-permeable hollow membrane into which molecules of neurotransmitter can diffuse down their concentration gradient. On the inlet side, the probe is connected, via fine tubing, to a microdialysis pump which perfuses artificial cerebrospinal fluid (ACSF) at a very low rate (in this experiment 2µl/minute) into the probe. On the outlet side, tubing carries the ACSF to collection vials clamped to the tether spring. As the ACSF meanders through the system, small molecules migrate from the extracellular fluid in the NAcc into the probe and out the other side for collection, although it should be noted that no net exchange of fluid takes place between brain tissue and probe (Joukhadar & Müller, 2005). Thus as the ACSF collects in the vials dopamine is simultaneously collected in this fluid for later quantification.

The experimental set-up was initiated a day in advance to allow the rats to adjust to their new surroundings, and thereby reduce the risk of confounding the results with unintended handling-induced stress elevations (most importantly a stress-induced dopamine elevation). Once

the subjects had been attached to the machinery, the flow of the microdialysis pump was adjusted to 0.2µl/minute and they were left overnight before the procedures of the following day. The equipment allows for the testing of up to 6 animals simultaneously.

On the day of experimentation, the apparatus was first examined in order to ensure correct fluid flow through the pump into the collection vials. Once checked, the flow of ACSF was then turned back up to 2µl/minute, allowing two hours for the complete readjustment of the flow rate; this time period allows any untoward accumulations of neurotransmitter within the microdialysis system to be flushed out. Then, once consistency of flow had been established across all subjects, sampling of ACSF began. Vials were changed every twenty minutes for three hours, with each full vial being replaced with an empty one, and the full vial immediately being placed on dry ice in a sealed container. This latter step prevents the rapid degradation of sample which may occur as a product of exposure to light and oxygen. The first hour's worth of samples were utilised to establish the baseline for each animal, after which point the remaining two independent variables were introduced: drug (cocaine or saline) and social cue (presence or absence of conspecific).

Cocaine hydrochloride (Sigma Chemical Company, St. Louis, Missouri) was dissolved in saline on the morning of treatment at a concentration of 2mg/ml, and rats were injected intraperitoneally[3] with 2mg/kg of body weight. The vehicle condition was a saline injection administered through the same route, the purpose of which was to ensure internal validity and eradicate concerns of history variation within subjects by controlling for the potential confound of injection-induced stress. For those animals in the 'with social cue' condition, a novel same-sex

[3] The intraperitoneal injection accesses the fluid surrounding the subject's intestines in the abdomen.

conspecific was placed in their box immediately after injection for 10 minutes of play, after which time they were removed. After this manipulation, the sampling continued as before for 2 more hours.

Behavioural Measure

Rats exposed to a social cue were observed and rated for aggressive behaviours on a scale of 1 to 5, with a low score indicating avoidance and a high score signifying aggression.

Probe Placement

Animals were administered a fatal dose of sodium pentobartital (Fatal Plus): males – 55mg in .6ml, females – 45mg in .5ml; their brains were then extracted for examination. The brains were flash-frozen with isopentane and stored at

-20ºC. Brains were then sliced on a cryostat, stained with cresyl violet dye and coverslipped in order to examine the location of the probe placement under a light microscope and confirm its position in the NAcc shell.

HPLC

The samples of ACSF were then subjected to detection using high performance liquid chromatography (HPLC), a chemical procedure that allows the investigator to identify and quantify neurotransmitter (in this case dopamine) molecules by a process of electrochemical detection using external standards. DA in the dialysates were determined using a Coulochem detector with an ESA 5014B analytical cell and an ESA 5020 guard cell connected to an ESA 80 x 3.2mm column which was maintained at 26ºC. The mobile phase was 150mM sodium dihydrogen phosphate monohydrate, 4.76mM citric acid, 3mM sodium dodecyl sulphate, 50µM EDTA, 10% methanol and 15% acetonitrile, pH=5.60. The potentials were set at -75 and

+250mV, and the guard cell potential was set at +250mV. Injections were performed with an ESA 542 autosampler using an injection volume of 35µl. Qualitative comparisons were run each day with external standards (Sigma).

CHAPTER III

ANALYSES

Sample

This thesis was a study of a continuous response between independent groups. Based on the results of pilot studies in our laboratory, we predicted that the response within each subject group would be normally distributed with standard deviation 0.8. If the true difference between group means is 1.5pg/µl of dopamine, 9 experimental subjects per group would be needed to be able to reject the null hypothesis that the population means of the independent groups were equal with probability (power) 0.95. The Type I error probability associated with this test of this null hypothesis is 0.05.[4] In order to account for procedural imperfections in the subjects, such as a misplaced probe, a buffer of 1 rat per group was added to ensure that sample demands were met. However, due to procedural difficulties, the final analysis comprised of the figures below:

Table I. Distribution of Subjects per Group.

	Group-Reared		**Isolate**	
	No Social	**Social**	**No Social**	**Social**
Saline	7	7	12	12
Cocaine	6	7	11	13

Statistical Analysis

This study was a mixed design, examining both the within-subjects repeated measure of time (i.e. samples 1-9 per animal) and the between-groups variables adolescent housing

[4] This information has been obtained from the website http://biostat.mc.vanderbilt.edu/PowerSampleSize.

condition, social cue and cocaine. A mixed ANOVA was therefore indicated, and where appropriate Fisher's LSD post-hoc tests were conducted as planned comparisons between groups. Simple effects of individual variables were also examined between the groups at each time point to determine any significant differences.

The analysis was based on the quantified dopamine samples, as detected by HPLC. The average of the first 3 samples was determined to establish a baseline value, and the percentage increase in dopamine from this value, as induced by experimental manipulation, was calculated for each subject. Group means for each time point were then determined, and significant differences between groups identified.

CHAPTER IV

RESULTS

Treatment with cocaine produced robust increases in extracellular dopamine. The analysis revealed a significant main effect of Drug, $F(8, 536)=11.61$, $p<.001$.

Exposure to social cue only did not produce significant increases in dopamine in any group.

The combination of cocaine treatment with housing condition did not reveal a significant interaction.

Exposure to the combination of cocaine and social cue produced a robust increase in DA, but only for isolate rats (Figure 3). There was a significant Social Cue x Drug x Housing interaction, $F(8, 536)=2.53$, $p<.05$. Post-hoc tests revealed a significant difference between the social cue and no social cue conditions for isolates receiving cocaine. No difference was observed between social conditions amongst group-housed animals receiving cocaine (Figure 2).

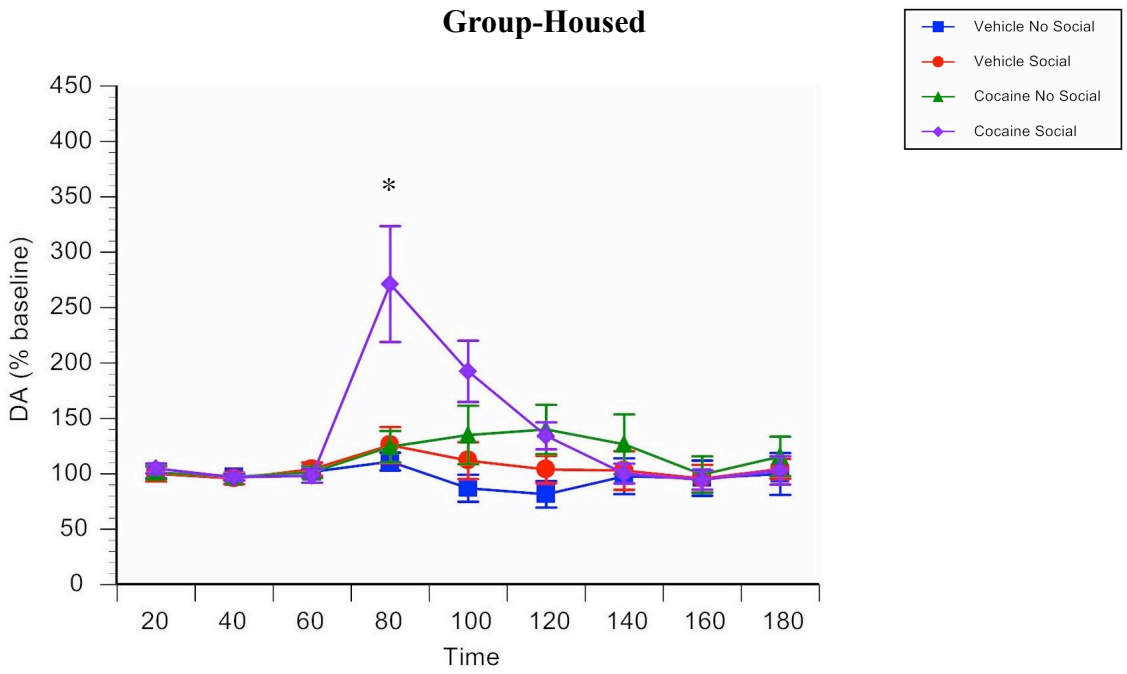

Figure II. Cocaine produced a robust increase in dopamine, with no significant difference between 'with social cue' and 'no social cue' conditions. Exposure to social cue alone failed to induce a significant increase of dopamine.

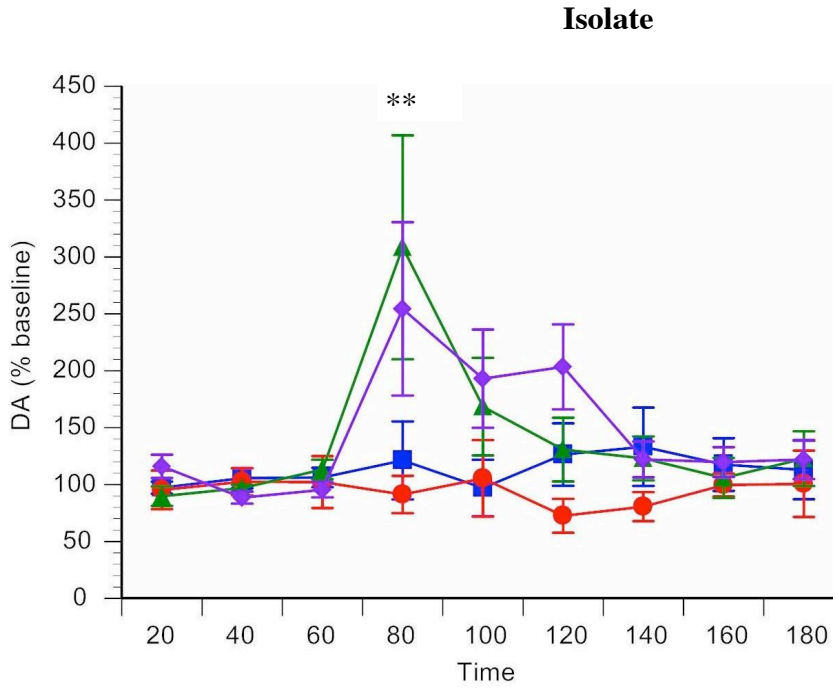

Figure III. Exposure to cocaine alone failed to produce a significant increase in dopamine, as did exposure to social cue alone. Increases in dopamine were produced only by the combination of drug and social cue.

* p<.05, groups receiving cocaine different from groups receiving vehicle.
** p<.01, isolates receiving cocaine and social cue different from all other groups.

Basal levels of dopamine were measured with no significant differences found between groups.

Table II. Basal levels of dopamine. Values are means ± SEMs.

	Group-Reared		Isolate	
	No Social	**Social**	**No Social**	**Social**
Saline	0.63 ± 0.11	1.11 ± 0.22	0.68 ± 0.18	0.89 ± 0.12
Cocaine	1.21 ± 0.24	1.20 ± 0.23	0.80 ± 0.18	1.09 ± 0.23

The expression of aggressive behaviour differed significantly amongst groups with social pairings. There were significant main effects of Drug, $F(1, 41) = 4.581$, $p<.05$, and of Housing Condition, $F(1, 41)=8.934$, $p=.005$. Post-hoc tests revealed greater aggression for isolate animals ($M=3.90$, $SD=.8539$) compared to group-housed animals ($M=3.10$, $SD=.68$); $t=3.41$, $p=.001$. Rats receiving saline ($M=3.87$, $SD=.91$) were also significantly more aggressive than rats receiving cocaine ($M=3.31$, $SD=.76$); $t=2.23$, $p<.05$ (see Figure 4).

Aggressive Behaviours

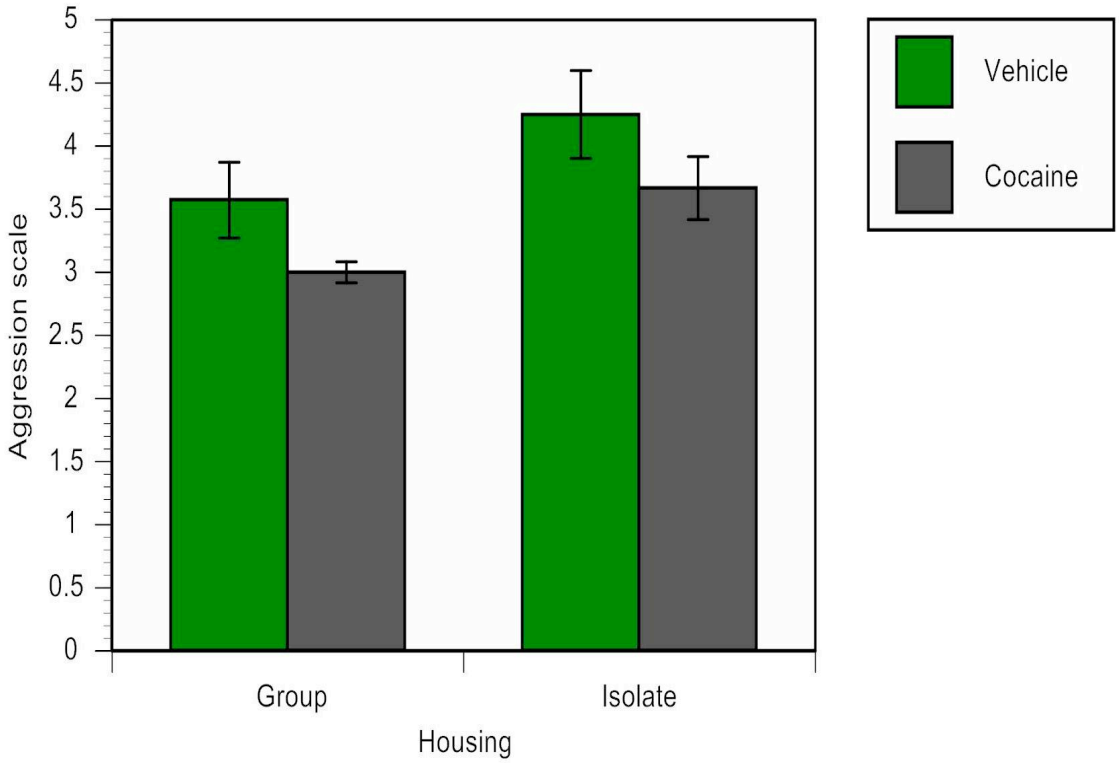

Figure IV. Significant main effects of housing and drug condition were observed. Isolate animals displayed significantly higher rates of aggression compared to group-housed animals. Also, animals receiving vehicle showed significantly higher rates of aggression compared to animals receiving cocaine.

CHAPTER V

DISCUSSION

For group-housed animals cocaine induced a significant increase in extracellular dopamine. These results are in accordance with much literature demonstrating a relationship between the drug and dopamine-mediated reward (for a review see Volkow, Wang, Fowler, Tomasi & Telang, 2011). This effect occurred for animals in both social cue and no social cue conditions. Similarly, our prediction of a synergistic interaction between cocaine and social cue was supported amongst isolates.

However, contrary to predictions, isolated animals in the drug and social cue condition did not display significantly higher increases in extracellular dopamine relative to all other groups. I originally hypothesised that both drug reward and social reward would be greater respectively for ASD animals; furthermore, I proposed that the interaction between these two rewarding stimuli would synergise and create a potentiating effect and that, stemming from the above predictions, the response to this interaction would be significantly greater for chronically stressed animals over group-housed controls. While I did in fact find an interactive effect between cocaine and social cue for animals in the ASD group, this was not reproduced amongst the group-housed animals. Moreover, the synergistic product of the drug and social cue pairing appears not to induce a significantly higher dopamine response as predicted, but rather serves to normalise the impaired response to cocaine only observed within the isolate group.

Some research suggests that isolation-reared animals display an increased vulnerability to both the locomotor effects and the rewarding properties of psychostimulants, though consensus is not universal. In terms of locomotion, a study by Weiss et al. (2001) found no significant

difference between isolated and control animals in response to an acute amphetamine administration, as measured by stereotyped behaviours, suggesting no behavioural change as a result of stress condition. Interestingly, however, differences were found between the groups in a chronic measure in which amphetamine was administered over the course of five days, with isolated rats displaying a reduced sensitivity to the locomotor effects of the drug. The findings of Weiss et al. raise the possibility that early life adversity may in fact serve as a protector against drugs of reward, at least in terms of their locomotor effects, though no conclusions can be drawn as to the subjective sensation of reward in this study. Also in contrast to the theory of stress-induced sensitisation, a study conducted by Phillips, Howes, Whitelaw, Robbins and Everitt (1994) found that control animals acquired cocaine self-administration more quickly than isolation-reared rats, seemingly offering support for the impaired response to psychostimulants found by Weiss et al. However, after five days of training this discrepancy was normalised between the isolates and controls, and may be better explained as a learning deficit rather than decreased drug-seeking behaviour.[5]

However, much other research does point to the sensitising effects of early chronic stress and its influence on drug-taking behaviour. In a study conducted by Howes et al. (2000) isolated rats demonstrated increased self-administration of cocaine over group-housed controls, and Mantsch, Saphier and Goeders (1998) found that treating animals with corticosterone reduced the latency to acquisition of self-administration of cocaine. It is not entirely clear whether social

[5] Early life adversity induces a range of neurological and psychological aberrations, including abnormalities in the PFC, a brain region heavily implicated in executive functioning, including working memory (Fone & Porkess, 2008). Thus the latency to self-administration acquisition may in fact be a product of a developmental neurological impairment.

isolation does in fact engender a heightened response to the drug, or whether isolated animals need more substance than controls, as to the best of our knowledge no break-point study has been conducted investigating social isolation and psychostimulants. Nevertheless, the change in response observed in the two above-mentioned studies is a pertinent issue as animals raised in the ASD model have a heightened reactivity of the HPA axis (Andersen & Teicher, 2009) and are thus more susceptible to mild environmental stressors, inducing the release of glucocorticoids which in turn can facilitate DA in the NAcc, bolstering the subjective reward of drugs of abuse. Preliminary data from our laboratory using microdialysis suggests that social cue-induced corticosterone in the striatum is facilitated in ASD rats. Thus the pairing of corticosterone with cocaine appears to more effectively reinforce the drug-taking behaviour. Indeed, the administration of corticosterone alone induces an increase in extracellular DA in the NAcc, and rats that are highly responsive to stress are more responsive to corticosterone, suggesting that high-stress rats may in turn be more sensitive to drug-induced DA reward (Piazza & Le Moal, 1996). This is echoed by a study by Brake et al. (2004) which found increased DA release in the NAcc in response to mild stressors in socially isolated animals, supporting the contention of increased HPA axis reactivity postulated by Andersen and Teicher (2009). Isolated rats in that experiment demonstrated greater sensitisation to the locomotor effects of cocaine, in contrast to the findings of Weiss et al. discussed above. Also, control rats raised normally had only 30% of the D3 receptor levels in the NAcc found in the isolate group, showing a much larger number of binding sites in the chronically stressed animals, and thereby suggesting a greater susceptibility to the rewarding properties of cocaine (Brake et al., 2004).

It has been postulated that animals exposed to chronic stress display a heightened level of basal DA relative to controls, and that this phenomenon may in part account for the sensitisation to drug reward produced by social isolation (Andersen & Teicher, 2009; Andersen et al.,1999; Higley & Linnoila, 1997). However, we did not find any significant differences between isolates and group-housed animals in the measurement of basal DA. In this our results are in accordance with Howes et al. (2000) in which study no differences were found in basal levels of DA between isolate and group-housed subjects. The hypothesis of abnormal basal levels of dopamine was thus not supported, but significant differences were observed between isolated and group-reared subjects regarding the *increase* of DA with respect to drug administration, with isolates showing greater sensitivity to the drug.

In another study, the number of DA transporters in the nucleus accumbens was reduced by 250% in rats that had been separated from their mothers during the early juvenile period (Meaney, Brake & Gratton, 2002). This reduction in transporters suggests that animals in this condition would be sensitised to the rewarding properties of psychostimulants as the reuptake mechanism is impaired, negatively influencing the clearing of DA molecules from the synapse. Interestingly, and in a similar vein, it has been demonstrated that rats raised in the ASD model also have a heightened response to food reward (Fone & Porkess, 2008; Lomanowska et al., 2011).

From results such as these we hypothesised that our rats raised in isolation would similarly display a heightened response to the presentation of the drug alone; this was not supported in our results as there was no difference amongst isolates with no social cue receiving cocaine or vehicle respectively. Similarly, we did not observe any significant differences in the

basal levels of DA between isolate and control groups. On the other hand, group-housed controls receiving cocaine only (no social cue) did in fact demonstrate a significant increase in DA in response to the reward stimulus compared to rats in the vehicle only condition. Thus the DA response to cocaine observed in the group-housed controls but absent in the isolates implies a blunted response to psychostimulants as a product of early chronic stress. Somewhat surprisingly, then, and in line with the findings of Weiss et al. (2001), early life adversity may serve as a protector against the rewarding nature of drugs of abuse when presented without other concurrent sources of reward, such as a social cue. However, as there is no clear conclusion on this topic more research must be done to establish the effects of ASD on psychostimulant reward.

Ongoing experiments in our laboratory suggest a trend that social play interaction is particularly rewarding for chronically isolated animals, with isolates displaying increased CPP for social cue over group-housed subjects. There is also an increased behavioural display of total social interaction amongst isolates, as well as a greater frequency of pinning, chasing and aggressive grooming. We measured aggressive behaviours in the social cue pairings during Timepoint 4, at which time either saline or cocaine was administered, and our results revealed, as expected, that there was a significantly greater demonstration of aggression amongst the isolates compared to group-reared rats. Interestingly, however, rats receiving cocaine showed a lower display of aggression compared to animals receiving saline. These findings are somewhat in line with a study conducted by Darmani, Hadfield, Carter and Martin (1990) who found that both acute and chronic administration of cocaine in the range of 10-20 mg/kg reduced aggressive behaviour in isolated mice. Doses in the 0.5-5 mg/kg range did not produce significant results in

an acute measure, however, but the discrepancy between their findings and ours may be related to the difference of species utilised.

In terms of dopamine response, for group-housed animals that received cocaine there was no significant difference between the social cue and no social cue conditions. The percentage increases observed in these groups fall well within the range of percentage increases found in other studies (see Zayara et al., 2011) and thus do not reflect a ceiling effect in which the interaction between social cue and drug could be obscured. Similarly, for group-housed rats with vehicle there was no difference observed between the social cue and no social cue conditions. These data support the notion that for normal controls social interaction is not particularly rewarding, though according to previous research social play is in fact an intrinsically pleasurable activity (Trezza, Baarendse & Vanderschuren, 2010). Somewhat surprisingly, psychostimulants such as cocaine actually reduce play behaviours[6] (Ferguson, Frisby & Ali, 2000; Homberg et al., 2007), yet social play has been shown to positively synergise with drugs of reward in terms of the behavioural response (Trezza, Baarendse & Vanderschuren, 2010). CPP data from our laboratory corroborate these findings, at least for ASD animals; male isolates demonstrated a strong preference for the side of the box in which social cue appeared both alone and in combination with cocaine. The preference observed amongst this group for social cue alone suggests that isolate animals find social exposure rewarding. However, we found no

[6] CPP data from our laboratory has supported this observation; behaviours analysed during conditioning trials have revealed a decreased number of pinnings in rats treated with cocaine. Interestingly, this quality of cocaine in suppressing play behaviours is attenuated by exposure to a non-treated conspecific. Ferguson, Frisby & Ali (2000) found that dyads of rats in which both animals were treated with cocaine displayed a significant reduction in pinning and crawl-overs compared to controls. However, this effect was lessened in dyads in which only one rat was treated with cocaine, suggesting that the sobriety of the other animal served to somewhat normalise this psychostimulant impairment. This phenomenon is of some interest for my experiment in terms of the valence of the interaction between experimental and stimulus rat, however a fuller discussion of this issue is beyond the scope of this paper.

increases in DA in the NAcc in response to social cue only in any of the groups, leading to the possibility that social reward is mediated by a pathway other than the mesolimbic DA system.

Serotonin has been implicated in the expression of social play, with induced increases of 5-HT causing a reduction of play behaviours (Homberg et al., 2007). As cocaine increases the availability of synaptic 5-HT as well as DA, it is thus possible that cocaine's play-reducing qualities may be linked more to its serotonergic action rather than its influence on dopamine. In terms of the subjective effect, however, the sensation of reward may be linked to activation of μ-opioid receptors in the NAcc (Trezza, Baarendse & Vanderschuren, 2010), rather than increases of dopamine in the NAcc. It has been proposed that dopamine's role in reward is motivation-based (Berridge & Kringelbach, 2008; Trezza, Baarendse & Vanderschuren, 2010); increases of DA in response to a pleasurable stimulus may indicate a learning process in which future motivation to acquire the reward will induce a corresponding increase in DA.

For group-housed subjects, the results showed no significant difference in DA response between rats with cocaine receiving either social cue or no social cue. Similarly, animals with vehicle receiving either social cue or no social cue showed no significant differences. This suggests that no dopamine-related learning occurred amongst the normally raised rats, perhaps because their continual exposure to opportunities of playful interactions in the home cage precluded the necessity of acquiring this association. By contrast, however, significant increases of dopamine were observed amongst animals receiving cocaine in both social cue and no social cue conditions. Rats were naïve to the drug prior to the day of experimentation, raising the possibility that dopamine-mediated learning did occur in response to the novel drug stimulus. As

no differences were observed between cocaine groups receiving social cue and no social cue respectively it is thus likely that the dopamine increase corresponds to the drug stimulus only.

Isolate rats, on the other hand, did not show an elevation of dopamine in response to the presentation of a social cue as expected, although it was a novel stimulus after four weeks of isolation. Similarly, there was a blunted response to the drug reward stimulus when presented individually which was only normalised by the synergistic interaction of the two variables. As discussed above, early chronic stress induces a range of cognitive deficits, including impaired learning, thus the failure of ASD animals to produce a DA response to either cocaine or social cue alone may be better explained by a learning abnormality rather than a lack of subjective reward.

The sensation of pleasure may instead be mediated by opioids rather than dopamine transmission; specifically, activation of μ-opioid receptors is indicated in the subjective feeling of reward (Trezza, Baarendse & Vanderschuren, 2010). Thus μ-opioid receptors may mediate the *hedonic* aspect of social play, whereas dopamine release may mediate its *motivational* or initial *learning* component. CPP data from our laboratory indicates behaviourally that social interaction is indeed rewarding for ASD subjects, and we thus predicted a greater increase in DA for chronically isolated animals in response to a social cue. This was not observed, however, thus potentially offering support for the above-mentioned theory. Our data suggest that the learning element of reward, mediated by the mesocorticolimbic DA pathway, is impaired by early chronic stress. Our CPP data suggests that the hedonic element is not disturbed; however, further discussion of the opioid systems and its role in reward is beyond the scope of this study.

In conclusion, then, the relationship between early chronic stress and the later development of cocaine addiction is not a straightforward one in which the presence of the former reliably produces the latter. Instead, environmental variables such as access to social cue modulate the appearance of drug sensitisation, and developmental stress may even serve as a protector against psychostimulant reward under some conditions. As the findings in the literature vary between studies, much research is needed to more clearly define the interaction between early stress, environmental conditions and drug reward, and to better understand the biological substrate which underpins it.

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